Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

SHOUT
SHARE
IT

U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 238.

B. T. GALLOWAY, *Chief of Bureau.*

THE MEASUREMENT OF THE OXIDASE CONTENT OF PLANT JUICES.

BY

HERBERT H. BUNZEL, PH. D.,

Chemical Biologist, Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations.

ISSUED MARCH 9, 1912.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1912.

BUREAU OF PLANT INDUSTRY.

Chief of Bureau, BEVERLY T. GALLOWAY.
Assistant Chief of Bureau, WILLIAM A. TAYLOR.
Editor, J. E. ROCKWELL.
Chief Clerk, JAMES E. JONES.

DRUG-PLANT, POISONOUS-PLANT, PHYSIOLOGICAL, AND FERMENTATION INVESTIGATIONS.

SCIENTIFIC STAFF.

Rodney H. True, *Physiologist in Charge.*

A. B. Clawson, Heinrich Hasselbring, C. Dwight Marsh, and W. W. Stockberger, *Physiologists.*
James Thompson and Walter Van Fleet, *Experts.*
Carl L. Alsberg, H. H. Bartlett, Otis F. Black, H. H. Bunzel, Frank Rabak, and A. F. Sievers, *Chemical Biologists.*
W. W. Eggleston, *Assistant Botanist.*
S. C. Hood, G. F. Mitchell, and T. B. Young, *Scientific Assistants.*
Alice Henkel and Hadleigh Marsh, *Assistants.*
G. A. Russell, *Special Agent.*

238
2

THIS PUBLICATION may be procured from the Superintendent of Documents, Government Printing Office Washington, D. C., at 10 cents per copy

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., October 23, 1911.

Sir: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 238 of the series of this Bureau the accompanying manuscript entitled "The Measurement of the Oxidase Content of Plant Juices," by Dr. H. H. Bunzel, Chemical Biologist, submitted by Dr. R. H. True, Physiologist in Charge of the Office of Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations.

In connection with the scientific investigation of a number of important technical processes and physiological and pathological conditions, it has been recognized that the activity of oxidizing enzymes is an important factor. While studying certain physiological phases of the curly-top of beets in connection with the Office of Truck-Crop Diseases and Sugar-Beet Investigations it became necessary to study these enzymotic relations as accurately as possible. Accordingly, Dr. Bunzel undertook to devise a practicable method of measuring oxidase activity. The present bulletin describes the resulting apparatus and methods and illustrates the manner of application with concrete data drawn from work on sugar beets, both normal and suffering from curly-top. It is believed that this method will prove a very valuable aid in investigating many diseased conditions in plants and will also facilitate a variety of other technical investigations having to deal with oxidases.

Respectfully,

B. T. Galloway,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

	Page.
Introduction	7
Short review of existing methods	9
Requirements and limitations of manometric methods	11
Description of the apparatus	13
Thermostat	13
Apparatus for temperature regulation	13
Fans for agitation of air	15
The heater	15
Cooling devices	17
Shaking apparatus	18
The thermometer	19
The oxidase apparatus	19
Titration apparatus	20
The switchboard	20
Illumination of the interior of the thermostat	20
Materials used in the experiments	21
Detailed description of the method suggested	24
Effect of the variable factors involved in the method on the total oxygen absorption	25
Effect of varying the concentration of pyrogallol	25
Comparative effectiveness of fresh and of old pyrogallol solutions	28
Effect of varying the concentration of potato juice	28
Effect of concentration of alkali in the absorption basket	29
Effect of varying the rate of shaking	30
Effect of varying temperature	30
Effect of shaking on the activity of the potato juice	31
Practical application of the method to the study of the curly-top of beets	33
Discussion of results	38

ILLUSTRATIONS.

PLATES.

		Page.
PLATE I.	Thermostat (closed)	12
II.	Thermostat (open)	14

TEXT FIGURES.

FIG. 1.	Detail of thermoregulator	14
2.	Switchboard	15
3.	Heater	16
4.	Shaking machine	17
5.	Aluminum clamps used on shaking apparatus	18
6.	Oxidase apparatus of the two-compartment type	19
7.	Oxidase apparatus of the three-compartment type	20
8.	Titration apparatus	21
9.	Diagram showing electrical connections	22

THE MEASUREMENT OF THE OXIDASE CONTENT OF PLANT JUICES.

INTRODUCTION.

The importance of the presence of oxidizing enzymes in plants is becoming more and more evident. The work of Palladin [1] and of others strongly emphasizes their fundamental rôle in the respiration of plants. Work done by Woods [2] in this Bureau bears out their significance in diseases of plants; furthermore, their causal relationship to color production in plants,[3] their important part in the darkening of tea,[4] as well as in that of bread during its making,[5] and in the production of the smooth, black, and hard lacquer of the Japanese from the white, fluid, soft secretion of the tree *Rhus vernicifera* [6] is well established.

[1] Palladin, W. Bildung der verschiedenen Atmungsenzyme in Abhängigkeit von dem Entwicklungsstadium der Pflanzen. Berichte der Deutschen Botanischen Gesellschaft, vol. 24, 1906, pp. 97-107.

Die Arbeit der Atmungsenzyme der Pflanzen unter verschiedenen Verhältnissen. Zeitschrift für Physiologische Chemie, vol. 47, 1906, pp. 406–451.

Ueber die Wirkung von Giften auf die Atmung lebender und abgetöteter Pflanzen, sowie auf Atmungsenzyme. Jahrbücher für Wissenschaftliche Botanik, vol. 47, 1910, pp. 431–461.

[2] Woods, A. F. The Destruction of Chlorophyll by Oxidizing Enzymes. Centralblatt für Bakteriologie, etc., pt. 2, vol. 5, 1899, pp. 745–754.

Observations on the Mosaic Disease of Tobacco. Bulletin 18, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1902, pp. 17–22.

[3] Agulhon, H. Influence de la Réaction du Milieu sur la Formation des Mélanines par Oxydation Diastasique. Comptes Rendus, Académie des Sciences, Paris, vol. 150, 1910, pp. 1066–1068.

Palladin, W. Synergin, das Prochromogen des Atmungspigmentes der Weizenkeime. Biochemische Zeitschrift, vol. 27, 1910, pp. 442–449.

Die Verbreitung der Atmungschromogene bei den Pflanzen. Berichte der Deutschen Botanischen Gesellschaft, vol. 26a, 1908, pp. 378–389.

Bourquelot, E., and Bertrand, G. Le Bleuissement et le Noircissement des Champignons. Comptes Rendus, Société de Biologie, Paris, ser. 10, vol. 2, 1895, pp. 582–584.

Bailey, I. W. Oxidizing Enzymes and Their Relation to "Sapstain" in Lumber. Botanical Gazette, vol. 50, 1910, pp. 142–147.

Bourquelot, E., and Fichtenholz, A. Nouvelles Recherches sur la Glucoside du Poirier, son Rôle dans la Production des Teintes Automnales des Feuilles. Comptes Rendus, Société de Biologie, Paris, vol. 69, 1910, pp. 605–607.

Combes, R. Du Rôle de l'Oxygène dans la Formation et la Destruction des Pigments Rouges Anthocyaniques chez les Végétaux. Comptes Rendus, Académie des Sciences, Paris, vol. 150, 1910, pp. 1186–1189

[4] Aso, K. On the Rôle of Oxidase in the Preparation of Commercial Tea. Bulletin, College of Agriculture, Tokyo, vol. 4, 1901, pp. 255–259.

[5] Boutroux, L. Le Pain et la Panification, Paris, 1897, p. 184.

Bertrand, G., and Mutermilch, W. Sur la Tyrosinase du Son de Froment. Bulletin, Société Chimique de France, ser. 4, vol. 1, 1907, pp. 837–841.

[6] Bertrand, G. Sur le Latex de l'Arbre à Laque. Comptes Rendus, Académie des Sciences, Paris, vol. 118, 1894, pp. 1215–1218.

Bertrand, G. Recherches sur le Latex de l'Arbre à Laque du Tonkin. Bulletin, Société Chimique de Paris, ser. 3, vol. 11, 1894, pp. 719–721.

These are only a few examples selected from the extensive literature on the subject,[1] but they fully demonstrate the great need of careful and thorough study of this class of substances.

Nearly all of the experiments attempting to correlate the oxidase content with biological processes in plants have been of a qualitative nature. In most of the cases the experimenters tried the effect of plant extracts with or without hydrogen peroxid on various organic compounds, such as guaiaconic acid, pyrogallol, hydrochinone, etc., and observed the changes in color. The plant juice or extract was then boiled, and by the negative result the enzymotic character of the active agent in the preparation considered was established. Then the effect of various poisons, such as hydrocyanic acid, arsenious acid, and sodium thiosulphate, was tried. From the results important conclusions were drawn and the research considered completed. These experiments demonstrate at least one very important fact, namely, that oxidizing enzymes are very abundant in the living plant forms examined.

The time has now come when mere qualitative study of enzymes is inadequate. This became particularly evident in the course of some biochemical investigations upon certain pathological conditions of important agricultural crops which were undertaken in this laboratory in cooperation with other divisions of the Bureau of Plant Industry. Among these conditions were the mosaic disease of tobacco, the curly-top of beets, and diseases of cabbage and spinach on the truck farms of Norfolk. For the first of these, Woods [2] long ago demonstrated changes in the oxidase mechanism. Work in this laboratory has raised the question whether the other conditions mentioned may not also present symptoms of this general type. It was found impossible to settle this question without determining the oxidizing power of these tissues and extracts quantitatively. Unfortunately no sufficiently accurate quantitative methods suitable for the purpose exist. It therefore became necessary to devise such. This task has been undertaken by the writer, and the present report is the first step in the solution of this problem.

---

[1] Issajew, W. Ueber die Malzoxydase. Zeitschrift für Physiologische Chemie, vol. 45, 1905, pp. 331–350.

Kelley, W. P. The Influence of Manganese on the Growth of Pineapples. Hawaii Agricultural Experiment Station, Press Bulletin no. 23, Honolulu, 1909, 14 pp.

Lagatu, H. Sur la Casse des Vins; Interprétation Nouvelle Basée sur le Rôle du Fer. Comptes Rendus, Académie des Sciences, Paris, vol. 124, 1897, pp. 1461–1462.

Bouffard, A., and Semichon, L. Contribution à l'Etude de l'Oxydase des Raisins. Son Utilité dans la Vinification. Comptes Rendus, Académie des Sciences, Paris, vol. 126, 1898, pp. 423–426.

Cazeneuve, P. Sur le Ferment Soluble Oxydant de la Casse des Vins. Comptes Rendus, Académie des Sciences, Paris, vol. 124, pp. 406–408.

Lépinois, E. Note sur les Ferments Oxydants de l'Aconit et de la Belladone. Journal de Pharmacie et de Chimie, ser. 6, vol. 9, 1899, pp. 49–52.

Lindet, L. Sur l'Oxydation du Tannin de la Pomme à Cidre. Bulletin, Société Chimique de Paris, ser. 3, vol. 13, 1895, pp. 277–279.

[2] Woods, A. F., op. cit.

SHORT REVIEW OF EXISTING METHODS.

The various methods, which in the past have been used in investigations of this sort, are briefly reviewed in an article by Foà.[1] Since many of the reactions accelerated by plant tissues or juices involve a change in color, various colorimetric methods have been used. Slowtzoff [2] measured amounts of laccase by the rate of coloration of Röhmann's [3] reagent (aqueous solution of paraphenylenediamine and metatoluilene). Brunn [4] followed the oxidation of guaiac resin colorimetrically; Euler and Bolin [5] recently modified this method for their own use; Medvedew [6] in the action of oxygen and aldehydase on salicylaldehyde determined the amount of the salicylic acid at various time intervals by a similar method. Kastle and Shedd [7] made use of the oxidation of the colorless phenolphthalin to the red phenolphthalein. Czyhlarz and Fürth [8] deserve credit for greatly increasing the accuracy of the colorimetric method. They oxidized the colorless leuco base of malachite green to the green dye and followed the formation of the latter by determining the extinction coefficient of the mixture from time to time.

As Foà points out, these methods are open to the criticism that the intensity of coloration is by no means necessarily proportional to the extent of oxidation of the colorless compound. Guaiac resin, for instance, assumes a blue color on oxidation which it loses when the oxidation is continued beyond a certain point.. The same author points out that the oxidases and peroxidases are not strictly specific in their mode of action, that the results obtained in the oxidation of one particular substance can not be generalized to the extent of determining the catalytic power with respect to other oxidations, that one of the requirements of a method is that it shall permit of the study of the oxidation of the various substances under investigation, and that the results obtained shall be strictly comparable.

In addition to the criticisms made by Foà, there are many other serious objections to the use of colorimetric methods in the measurement of oxidizing enzymes. In the first place the tissue extracts available are seldom clear and colorless, but are generally grayish and

[1] Foà, C. Eine Methode graphischer Registrierung einiger Gährungsvorgänge. Biochemische Zeitschrift, vol. 11, 1908, pp. 382–399.

[2] Slowtzoff, B. Zur Kenntniss der pflanzlichen Oxydasen. Zeitschrift für Physiologische Chemie, vol. 31, 1900–1, pp. 227–234.

[3] Röhmann, F., and Spitzer, W. Ueber Oxydationswirkungen thierischer Gewebe. Berichte der Deutschen Chemischen Gesellschaft, vol. 28, 1895, pp. 567–572.

[4] Brunn, J. Die Verwendung der Guajakmethode zur quantitativen Peroxydasenbestimmung. Berichte der Deutschen Botanischen Gesellschaft, vol. 27, 1909, pp. 505–507.

[5] Euler, H., and Bolin, I. Zur Kenntniss biologisch wichtiger Oxydationen. III Mitteilung. Zeitschrift für Physiologische Chemie, vol. 61, 1909, pp. 72–92.

[6] Medvedew, A. Ueber die Oxydationskraft der Gewebe. Archiv für die Gesammte Physiologie, vol. 65, 1897, pp. 249–277.

[7] Kastle, J. H., and Shedd, O. M. Phenolphthalin as a Reagent for the Oxidizing Ferments. American Chemical Journal, vol. 26, 1901, pp. 526–539.

[8] Czyhlarz, E. von, and Fürth, O. von. Ueber tierische Peroxydasen. Beiträge zur Chemischen Physiologie und Pathologie, vol. 10, 1907, pp. 358–389.

turbid, due, among other things, to the partial oxidation and subsequent precipitation of the chromogens contained in them. Since artificially prepared color standards are free from colored suspended matter, the comparison becomes very difficult and at the best inaccurate. On the other hand, if the oxidase-containing solutions are freed from such disturbing constituents or if only very small amounts of the juice to be studied are used, the methods based on color comparison become very unreliable. What is badly needed is a method applicable to the juice or extract freshly prepared from the plant tissue, the accuracy of which will be enhanced rather than impaired by the use of larger quantities of material. Fresh plant juices always contain appreciable amounts of protein in solution. It is well known that all proteins, being amphoteric colloids, are capable of combining with or absorbing colored compounds of all sorts. Since only a small amount of the colored substance is found in the manipulations previously referred to an appreciable error is introduced whenever fresh tissue juice is used, especially since the protein-dye combination is frequently insoluble.

Methods based on the measurement of the quantity of precipitate produced in the course of oxidizing water-soluble substances to insoluble compounds are more reliable, but very limited in their applicability. Fürth and Jerusalem [1] measured the tyrosinase content of mushrooms by the volume of melanin precipitate produced; Bach and Chodat's method,[2] which is based on the weighing of the purpurogallin formed in the presence of hydrogen peroxid and peroxidase is so well known that it does not require description. The first method here mentioned is inaccurate, the second tedious, owing to the number of weighings, and neither is of general applicability.

As Foà points out, the methods most satisfactory for the measurement of the rate of reactions involving oxygen absorptions are those in which the quantities of oxygen absorbed are determined by measuring the changes of pressure within the reaction flask. This paper deals with the description of such a method.

A manometric method has been devised and used successfully by A. P. Mathews in his work on the spontaneous oxidation of the cell constituents.[3] Similar methods have also been in use by many other observers for the sake of obtaining a measure of the respiratory enzymes present, but no one has observed all of the precautions nec-

[1] Fürth, O. von, and Jerusalem, E. Zur Kenntniss der melanotischen Pigmente und der fermentativen Melaninbildung. Beiträge zur Chemischen Physiologie und Pathologie, vol. 10, 1907, pp. 131–173.

[2] Chodat, R. Darstellung von Oxydasen und Katalasen tierischer und pflanzlicher Herkunft. Methoden ihrer Anwendung. In Abderhalden, E. Handbuch der Biochemischen Arbeitsmethoden, Berlin, 1910, vol. 3, pt. 1, pp. 42–74.

[3] Mathews, A. P. The Spontaneous Oxidation of the Sugars. Journal of Biological Chemistry, vol. 6, 1909, pp. 3–20.

Mathews, A. P., and Walker, Sidney. The Action of Cyanides and Nitriles on the Spontaneous Oxidation of Cystein. Journal of Biological Chemistry, vol. 6, 1909, pp. 29–37.

essary in such measurements. The precautions to be observed, as well as the drawbacks of all methods of this type, are described below.

One of the main reasons for choosing this means of measuring oxidases was the following: It is generally thought that the oxidases in their mode of action are purely catalytic—i. e., that they accelerate reactions without being consumed in the process, and, furthermore, that the quantity of material transformed in the process is in no simple proportion to the quantity of oxidase used. If the oxidases are enzymes in this sense of the word, the only correct means of estimating them is to measure the degree to which they can accelerate certain oxidations. The rate of such an accelerated reaction would necessarily be a measure of the concentration of the enzyme present, and to determine the rate under known and uniform conditions would be to measure the strength of the oxidase preparation used. By means of the method described in this bulletin the rates at which the oxidations take place can be followed with great ease. If the reaction is one of strictly catalytic nature, the method will be satisfactory, since measurements of the quantity of oxygen absorbed can readily be made at any time in the course of the experiment. However, one of the first facts that became apparent in the course of this investigation was that the oxidase is not a catalyst in the sense just described, because a definite quantity of the oxidase preparation was found to oxidize only a definite and relatively small quantity of pyrogallol. Therefore the relations between the strength of the oxidase preparation, the amount of the oxygen absorption, and the rate of the absorption must all be thoroughly studied. The present paper has been limited necessarily to the examination of the total oxygen absorption only, while the study of the rate of this absorption has been left for a later time.

REQUIREMENTS AND LIMITATIONS OF MANOMETRIC METHODS.

It is obvious that in a satisfactory manometric method for the determination of oxidases the following conditions must be fulfilled:

(1) The temperature at which the reaction is carried out must be nearly constant—i. e., it should not vary more than 0.10 of a degree. This is an absolutely essential condition, since the pressure within the flask in which the experiment is carried out is directly proportional to the absolute temperature. At 37° C. (t), for example, or 310° (T), a change of 1 degree will be accompanied by a rise or fall in pressure of 0.25 of a centimeter. If, according to the requirement suggested, the temperature rises no more than 0.10 of a degree, the pressure will fluctuate no more than 0.025 of a centimeter which is the accuracy with which the manometric readings can be made.

Another reason for performing all experiments at a fixed temperature is the well-known variation of the rate of chemical reactions

with variations of temperature. In most reactions studied, a change of 1 degree alters the velocity of the reaction about 20 per cent; a change of 0.10 of a degree will therefore introduce an error of not more than 2 per cent, and, since the mean temperature fluctuates a great deal less than 0.05 of a degree, the error will be even less.

(2) In all experiments in which oxidation is carried out by means of the oxygen derived from the air, only the oxygen in the solution can take part in the reaction. The velocity of the reaction according to the mass law is, among other things, proportional to the concentration of the dissolved oxygen. The concentration of the latter can be made constant in all experiments if the following conditions prevail: (*a*) The partial pressure of the gaseous oxygen in the reaction flask should be constant throughout any experiment and the same in all of the experiments. (*b*) The reaction of the solution should be the same in all experiments. (*c*) The temperature within the flasks should be always the same. (*d*) The liquid should be saturated with oxygen under the standard conditions, *a*, *b*, and *c*, and kept saturated throughout the experiment.

(3) It is essential that standard conditions should prevail before the reaction begins. By standard conditions is meant that the gas in the flask should be of known composition, at the temperature at which the measurements are to be made, and that it should be saturated with water vapor, so that any change in the density of the gas or gas mixture in the reaction vessel in the course of the actual measurements will be due entirely to oxygen absorption and not to change in temperature and moisture content.

(4) The change in pressure as indicated by the manometer should be entirely due to oxygen absorption, and not to the difference between the oxygen consumed and the carbon dioxid produced, which condition necessitates that the carbon dioxid be removed from the gas mixture in the reaction vessel as fast as it is formed.

(5) The amount of carbon dioxid produced in the experiments should be measured. The total carbon dioxid production may serve as a check on the rate and extent of the oxygen absorption.

(6) In all of the experiments the rates of oxidation should be measured. If the reaction comes to completion within several hours or a few days, the total quantity of oxygen absorbed must be determined.

A method of this sort has several disadvantages. In the living cell a great variety of combustible substances are always present at different stages of oxidation. These substances hold the oxygen they contain with different degrees of firmness, and are ready to give it up to other substances less "eager" to throw it off, i. e., having a lower oxidation potential. In this way an easily oxidizable substance in solution can obtain its oxygen not only from the air above

THERMOSTAT (CLOSED).

the solution but also from other substances present in the same solution. The latter phase of the oxidation is not taken into account in methods based on pressure differences occurring in the reaction vessel.

Another possible source of error is the shaking involved in the experiments. It has been shown in the case of many enzymes that they are destroyed on violent shaking of their solutions. In the experiments to be herein described the shaking was quite gentle and did not last more than a few hours, so that actual destruction of the enzymes is not to be anticipated. The effect of the shaking on the oxidizing power of the potato juice was investigated and is discussed further on in this bulletin.

DESCRIPTION OF THE APPARATUS.

Thermostat.—The thermostat used in these experiments is shown in Plates I and II. The inner dimensions of the box within which the temperature was maintained constant are 138 centimeters long, 38 centimeters deep, and 55 centimeters high. The walls of the box, from without inward, consist of $\frac{3}{4}$-inch oak, a few layers of paper, a layer of $\frac{1}{4}$-inch composition board, and a thickness of $\frac{1}{8}$-inch asbestos wood. The ice box *I*, which is 56 centimeters long, 37 centimeters deep, and 40 centimeters high, was in addition lined with a 1-inch layer of cork and a sheeting of galvanized iron. As can be seen from the illustrations, the thermostat proper, as distinct from the ice box, has two front doors which slide tightly in separate grooves and are supported by steel springs. The inner door, Plate II, has in its upper half two glass panels, of which the right one, *a'*, slides to the left, while the outer door has only one glass panel, *b'*, which is on the right and also slides. Over this panel slides another one of wood. The glass window on the outer door is made of orange glass, to absorb most of the actinic rays. This was designed to facilitate experiments concerning the effect of light on the reactions involved. For the same purpose the wooden panel provides means to eliminate all light from the interior of the box. By means of this arrangement of windows it is possible to get at the inside of the box and carry out operations there, such as the closing of stopcocks, etc., without perceptibly altering the temperature, since it is necessary to slide the windows only enough to permit the introduction of the experimenter's hand.

Apparatus for temperature regulation.—The customary method of temperature regulation with electric heating was used. The thermoregulator (fig. 1) consists of a tube about 14 meters in length, bent as shown in the figure, and mounted on a wooden frame. The frame is fastened to the wall in such a way that a space of about 3 centimeters is left between the glass tube and the wall, thus providing free circulation of air around all parts of the thermoregulator. The glass tube is thin walled and has an inner diameter of about 2 milli-

meters. No difficulty is experienced in using thin-walled tubing, since the long tube is supported by means of wire at each one of the bends, and the weight of mercury supported by a single perpendicular section of the tube is only about 10 grams. One end of the tube being closed, the mercury has to rise and fall in the straight portion, into which the nickel wire *A* may be moved up and down. Once set, it is held stationary by the friction in the rubber tube *R*. The circuit energizing the electromagnet of the relay is made and broken at the lower tip of this nickel wire.[1] The other contact on the thermoregulator is made on the other end of the cup *B* filled with mercury, connection being established between the mercury in the cup and the mercury in the long tube by means of a short piece of platinum wire, *C*.[2] The relay (fig. 2) *N* is 150 ohms. The current required to energize the electromagnet is furnished by the dry cells,

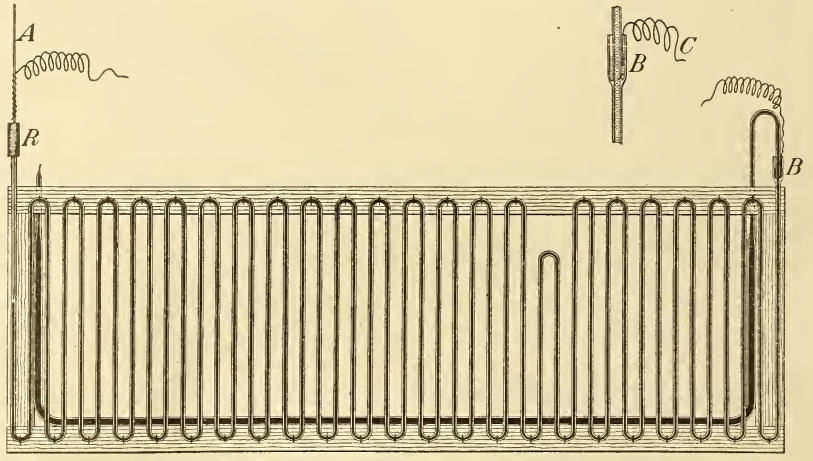
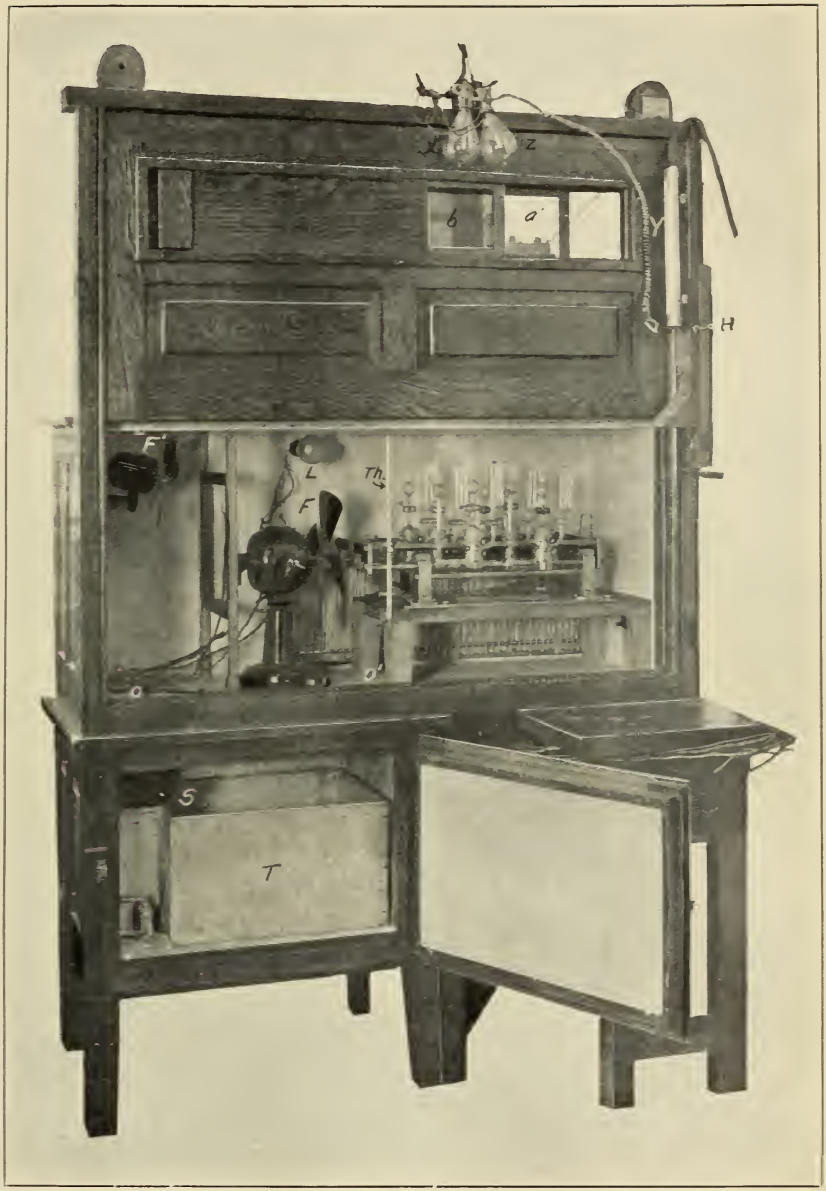


FIG. 1.—Detail of thermoregulator.

Plate I, *C*, of which there are two series of three in parallel. The batteries are thrown into the relay-thermoregulator circuit by means of the switch, figure 2, *c'*. If some disturbance due to the batteries should arise in the middle of the experiment, switch *c'* is turned off and switch *k'* turned on. Then the regular lighting circuit furnishes the current, the resistance, *R*, being used to step down the voltage to about one-tenth of its value. The resistance of the coils being 150 ohms, only about 0.02 of an ampere passes through the thermoregulator under ordinary conditions. The sparking under these circumstances is very slight indeed, but was reduced still more

[1] The use of nickel in this connection was recommended by Mr. T. B. Freas, of Columbia University, who uses nickel contacts in most of his thermostats. The nickel has an advantage over platinum, inasmuch as mercury does not hang on it as it does at times on platinum.

[2] Gore, H. C. Studies in Fruit Respiration. Bulletin 142, Bureau of Chemistry, U. S. Dept. of Agriculture, 1911.



THERMOSTAT (OPEN).

by means of the condenser *K*. Another condenser, *K'*, is used across the contacts made on the relay.

Fans for agitation of air.—On account of the low specific heat of the medium, it is necessary to agitate the air within the box very thoroughly during the experiments. This is done by means of two fans. The small fan, Plate II, *F'*, mounted right behind the heater, is used in the beginning of each experiment until the interior of the box has reached the temperature desired. Then this fan is stopped and the large (12-inch) fan *F*, in the middle of the box, is started. This keeps the temperature throughout the box constant within 0.1 to 0.2 of a degree.

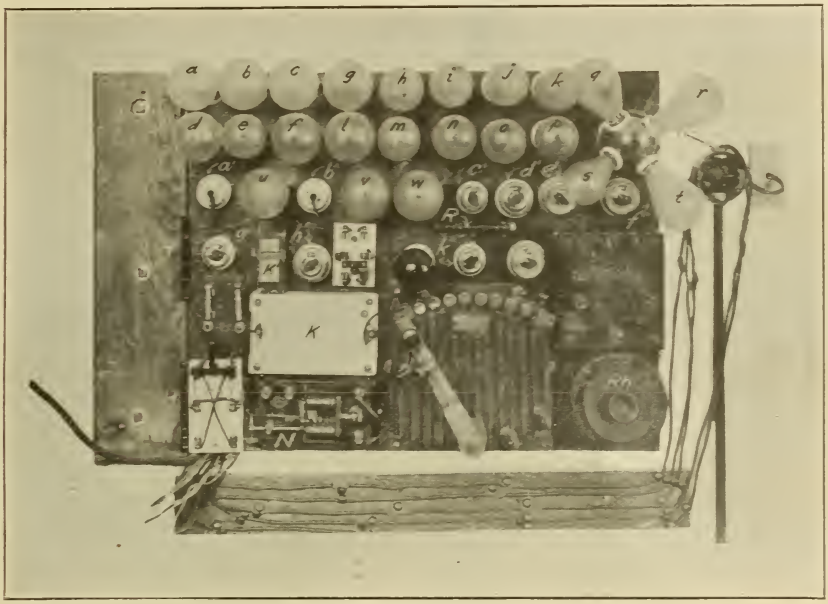


FIG. 2.—Switchboard.

The heater.—The heater (fig. 3) consists of a frame made of ½-inch ebony-asbestos composition. The frame has the outer dimensions 12 by 12 inches and a width of 1 inch throughout. The wire used for the heating is drawn tightly through the rings fastened on the perpendicular sides of the frame. The wire is nichrome wire No. 23, of about 0.025 of an inch in diameter. About 40 feet of wire were used, making the resistance of the heater 44 ohms. The current passing through the heating wire can be raised in very small steps within very wide limits. By means of a switch, figure 2, *h'*, the whole current can be sent through the wire. By means of switch *c'* the lamps *a*, *b*, *c*, *d*, *e*, and *f*, which are all mounted in parallel, are thrown in series with the heater. If the six lamps are found to allow too little current to pass through the heater, the ten lamps *g*, *h*, *i*, *j*, *k*, *l*, *m*,

n, *o*, and *p* can also be thrown in parallel with the others by means of the switch *i'*. Since any of these lamps can be cut out, and since 8, 16, or 32 candlepower lamps can be used in any of the sockets, a great variation in the resistance of the heating circuit can be attained. Moreover, this means of varying the current has the advantage that one knows at a glance what the actual current passing through the heater is. By means of this arrangement it was possible to heat up the interior of the box very rapidly to any temperature desired and to supply it with sufficient heat to maintain the temperature a little below that at which the experiments were carried on. The difference between the temperature of the box and the actual temperature of experimentation was controlled by means of the four lamps *q*, *r*, *s*, and *t*, the switch *f'*, switch *c'*, the relay, and the thermoregulator. The current of this intermittent heating device was also variable, since any of the lamps could be replaced by others of any size.

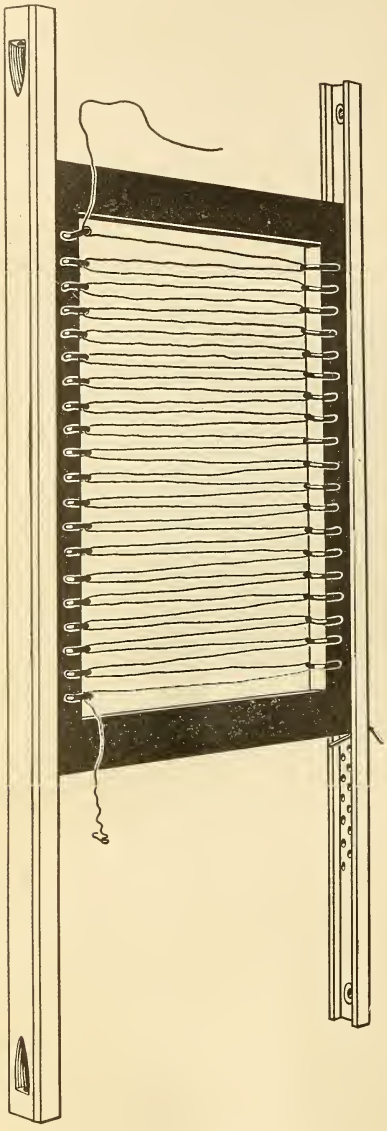


FIG. 3.—Heater.

This arrangement has a number of advantages. The current passing through the relay contacts is very small and therefore hardly any sparking is produced at break and make. Only very little additional heat is supplied per unit of time, so the overheating due to noninstantaneous mixing of the air and the lag of the mercury in response to the increased temperature is reduced to a minimum. The same holds true for the undercooling. The results are minimal fluctuations of temperature and a uniform temperature throughout the thermostat.

Cooling devices.—If the experiments are to be carried on at or below room temperature, cold air is blown into the box. The air is cooled in the ice chest, Plate I, *I*, and blown into the thermostat through the opening, Plate II, *O*. This opening can be closed by means of a sliding board. The warm air returns through a smaller opening, *o'*, at the opposite end of the ice box, which hole can be closed in a similar way. A small blower, Plate II, *S*, is fitted into the opening in the ice box and supplies cold air whenever the temperature in the box rises above the temperature desired. Vice versa, when the temperature has fallen somewhat below that desired, the fan motor stops and the heater comes into action. This is accom-

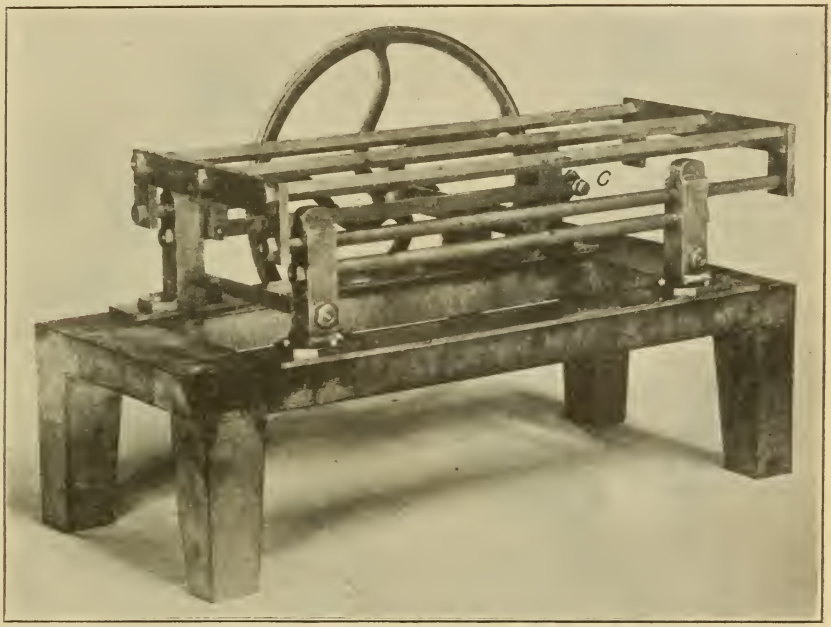


FIG. 4.—Shaking machine.

plished by use of the relay. When the relay closes the main circuit, the fan motor is in parallel with the heater. The circuit passes through the four lamps in parallel, *q*, *r*, *s*, and *t*, and most of it will pass through the heating wire, since the resistance of the latter is only 44 ohms and that of the motor about 1,000 ohms. Under these circumstances not sufficient current passes through the fan motor to set it in motion. As soon as the circuit in the relay is broken, however, all of the current passes through the motor and cold air is blown into the box and the heating practically stopped simultaneously. In the same circuit with the blower are the two lamps *v* and *w*, which are in parallel and by means of which the speed of the blower can be varied. By means of switch *b'* the blower can be put

out of the circuit. The tank, Plate II, *T*, is filled with ice or salt and ice according to the temperature desired.

For the lowest temperatures obtainable it is more satisfactory not to use the heater at all. Under such circumstances the fan motor is shunted with the 10 lamps *g*, *h*, *i*, *j*, *k*, *l*, *m*, *n*, *o*, and *p*, which are thrown in parallel with the fan by throwing the switch *i'* to its lower position. The resistance of the 10 lamps of 32-candlepower is 44 ohms, which is very much less than the resistance of the fan motor.

It is suggested that this principle of thermostat construction solves one of the problems with which a tropical laboratory has to contend. It makes it possible to keep constant temperatures below that of the environment with a relatively small consumption of ice.

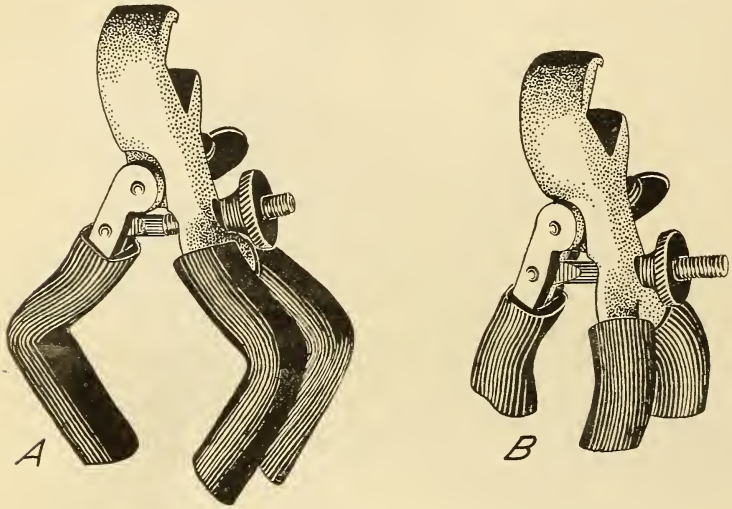


FIG. 5.—Aluminum clamps (*A* and *B*) used on shaking apparatus.

Shaking apparatus.—The shaking machine shown in figure 4 was designed for this special purpose. On account of the detailed illustration little description is necessary. Its main advantages over the shaking machines on the market are (1) the tight bearings which make any up and down motion impossible, (2) the crank, *C*, at the end of the shaft, whereby the extent of the excursion can be varied, and (3) the nature of the carriage on which nine or even more oxidase apparatus can be clamped at one time. The clamps, *A* and *B*, shown in figure 5, are of aluminum and fit rigidly on the square bars of the carriage. By means of the four pulleys, Plate I, *P*, and two pulleys on the motor, it is possible to vary the rate of shaking for 10 complete excursions from 4 to 12 seconds. The intermediate speeds not obtainable by means of the pulleys are obtained by means of the rheostat, figure 2, *Rh*. Only one belt is required, since the position

of the motor is adjustable. It is mounted on a board which can be moved backward and forward on wooden rails by means of a lever and can be made fast in any position with a pin.

The thermometer.—The thermometer, Plate II, *Th.*, clamped on the carriage with a small special clamp, figure 5, *B*, indicates the temperature within the box.

The oxidase apparatus.—The apparatus in which most of the experiments were carried out is shown in figure 6. It has a volume of about 150 cubic centimeters. The indentation *D* divides the bottom part into two compartments, *A* and *B*. Liquid may be filled into *A* from the 2-cubic-centimeter burette, *F*, through the stopcock *C*. The compartment *B* may be filled out of the bulb *G*, which has a volume of 8 cubic centimeters, through the stopcock and tube *L*, or by separating the ground joint *K*. The tube *L* supports a small glass basket, *H*, holding about 10 cubic centimeters. This basket is provided with a rim around the top to prevent any liquid from splashing out when the apparatus is being shaken. It is also sufficiently elevated from the bottom of the flask to prevent any liquid from splashing in. This precaution of course is quite essential, since the oxygen absorption due to any mixing of pyrogallol and alkali would make the manometric readings useless. The manometer *M* is graduated to millimeters. It may be disconnected by closing the stopcock *I*. The apparatus is well flattened on the bottom and stands firmly. The part holding the basket can also be made to stand firmly on the bottom of the basket.

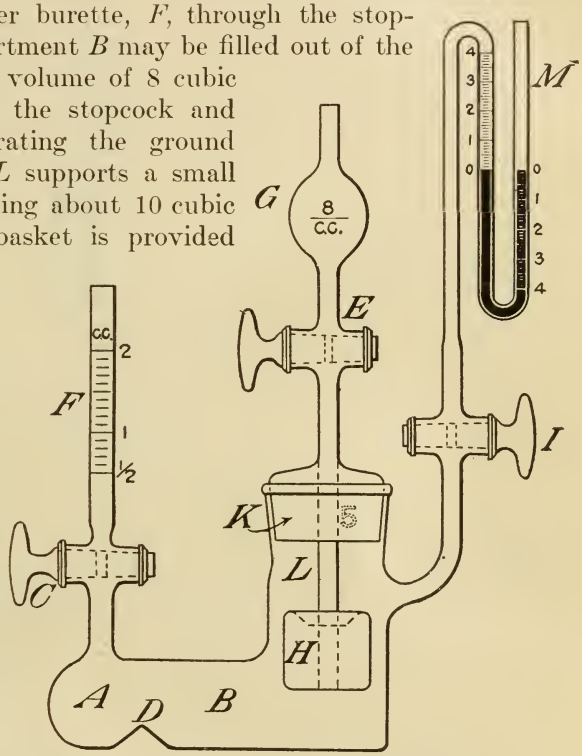


FIG. 6.—Oxidase apparatus of the two-compartment type.

Another type of absorption flask was also used (fig. 7). This was provided with two indentations and had, therefore, three compartments of approximately equal size.

The oxidase apparatus used were not all of exactly the same size, as it is difficult to blow all uniformly. In all of the experiments

described in this bulletin, only the six numbers specified were used. Their sizes are as follows: No. 1, 143 cubic centimeters; No. 4, 153; No. 5, 152; No. 7, 156; No. 11, 144; No. 12, 133.

Titration apparatus.—For the purpose of titrating the contents of the basket, the inner part of the ground joint of the titration apparatus (fig. 8) was removed and transferred to a flask in which all titrations were carried on in a carbon-dioxid-free atmosphere. The bottom of the flask was covered with a 30 per cent sodium hydroxid solution. The joint holding the basket may be held in place by the large-bore rubber stopper *R*. The burette *B*, graduated to tenths, can be rotated in the ground joint *G*, so that the tip will be just above the basket when in place. The small bulb *C* is fastened to the burette by a ground joint and filled with cotton to keep dust out of the solution.

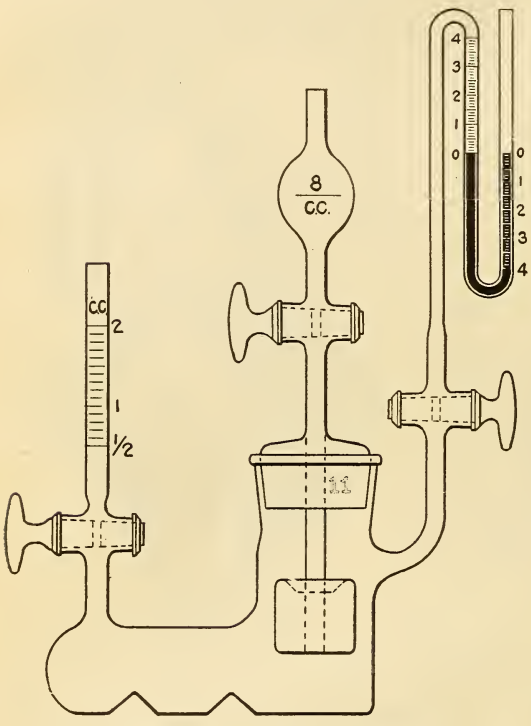


FIG. 7.—Oxidase apparatus of the three-compartment type.

The switchboard.—The switchboard on which all of the lamp resistances and switches are mounted is shown in figure 2. If changes in wiring are to be made, one can get at the back of the board very readily by swinging it around on the hinges, *H*. Moreover, the whole board can be removed if the wires to and from it are disconnected. The diagram (fig. 9) shows all of the connections. The mode of operation has been described in connection with the heating arrangement.

Illumination of the interior of the thermostat.—The lamp, Plate II, *L*, serves to illuminate the interior of the box while preparations for experiments, such as the clamping on and filling of the oxidase apparatus, are being made. After the shaking has begun, this lamp is no longer used, and thus unnecessary changes in temperature may be avoided. Whenever readings are to be made, the lamp, Plate I, *Y*, is used. This lamp is one foot long, incandescent, and mounted on

a reflector. When not in use, it can be thrown out of position, as shown in Plate II. When readings are to be made, the hook, Plate II. *H*, is released, and the lamp drops into position, as shown in Plate I. and throws light through the windows. The intensity of the light can be varied by means of the lamps, Plates I and II, *y* and *z*.

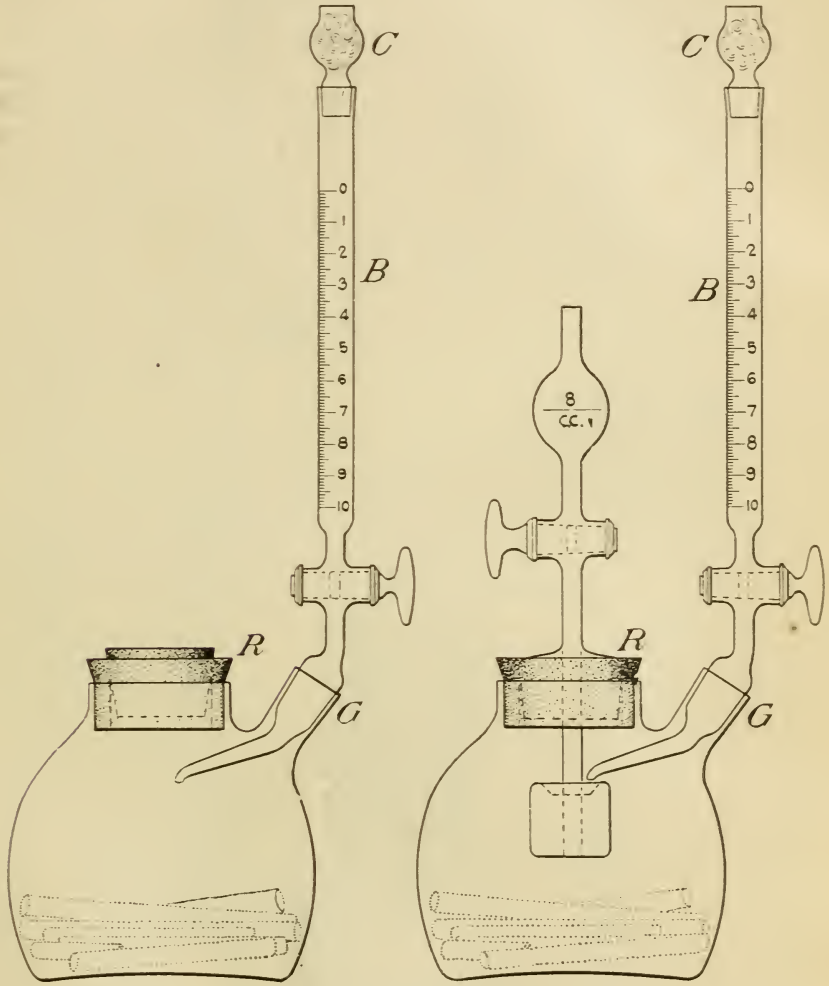


FIG. 8.—Titration apparatus.

MATERIALS USED IN THE EXPERIMENTS.

In most of the experiments described in this paper, potatoes furnished the oxidase preparations. These were used for a number of reasons. They are easily obtainable at all times of the year, and can be readily grown for experimental purposes if it is desirable to study the variation of oxidase content with varying conditions. Numerous experiments by other observers show that potatoes are rich in

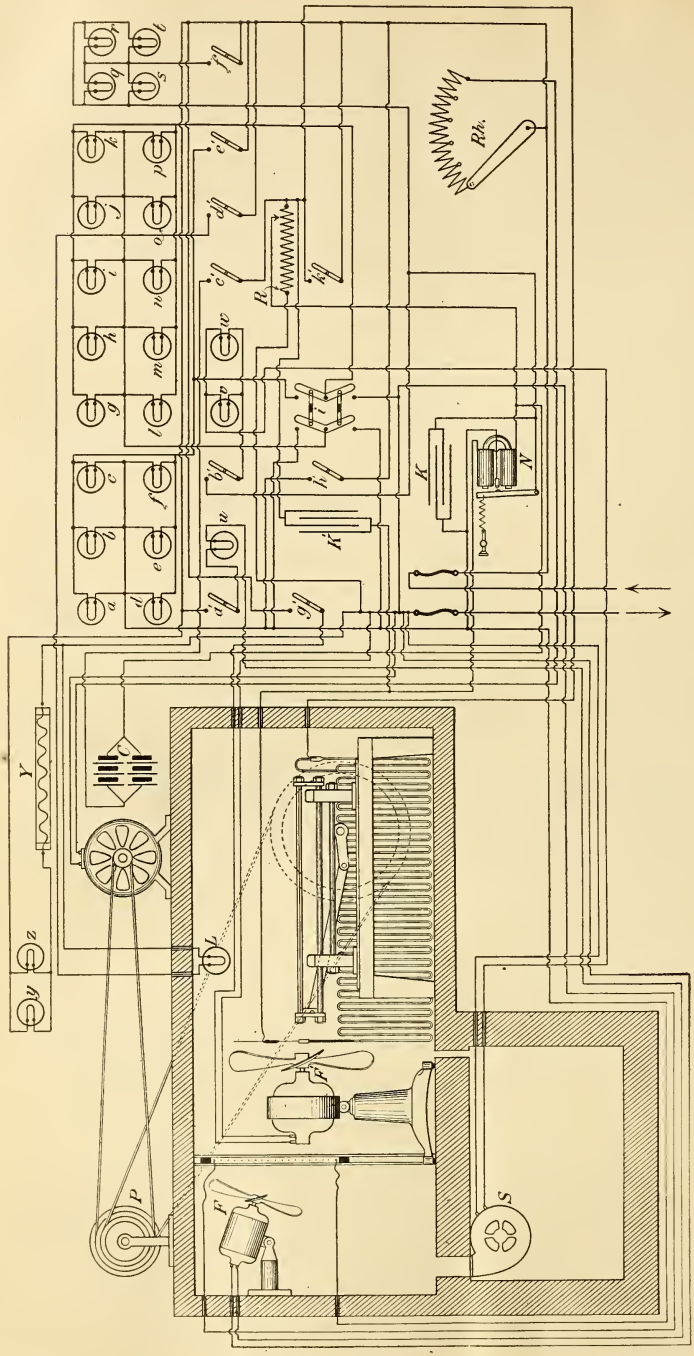


FIG. 9.—Diagram showing electrical connections.

oxidases. They seemed, therefore, the best test object for elaborating the method.

The potatoes were rinsed off with cold water and wiped dry with a clean towel. They were peeled, and the peelings were ground up in a meat chopper. The juice was obtained by pressing the pulp through a piece of silk cloth. In all the experiments only fresh juice was used. The juice of beet leaves was obtained by pressing it from the cleansed leaves after grinding them in a meat chopper. Neither the potato nor the beet juice was filtered through paper. Since the activity of the juice undoubtedly depends to some extent on the mode of preparation, a method will be worked out in the near future by which uniformity in this process can be assured.

To start with, it was decided to use pyrogallol as the substance to be oxidized. There were several reasons for choosing this substance: It can be obtained in a high degree of purity, so that results can be duplicated at any time, it is easily soluble in water, and its solutions are very nearly neutral. Since the only satisfactory method for the measurement of peroxidases—that of Bach and Chodat—is also based on the oxidation of pyrogallol, it should be possible to determine in the same sample of plant juice the relationship between the acceleration of the oxidation by oxygen and that by hydrogen peroxid. This was another reason for the use of pyrogallol in these experiments. More important than all the others, however, is the following reason: Until the mode of action of the oxidases is better understood it is best to assume that their action is analogous to that of other enzymes, i. e., purely catalytic. According to such an assumption they are incapable of initiating a reaction, but accelerate certain reactions which go on in their absence at a very much slower rate. If, therefore, it is desired to study the catalytic effect of the oxidizing enzymes, they must be allowed to act on a substance which is also oxidized in their absence, but very much more slowly. The relative speed of the reaction with and without plant juice present would express the enzymotic activity of the juice. Such a reaction is the oxidation of pyrogallol by atmospheric oxygen.[1] If a solution of pyrogallol is allowed to stand in the open air, it will assume a yellow tinge in several hours, which will deepen and then, passing through orange, gradually become a deep red. These color changes are accelerated if air or oxygen is passed through the solution constantly, or if hydrogen peroxid is added, or still more if hydrogen peroxid is added and air is passed through at the same time. If the access of oxygen is entirely prevented, the pyrogallol solution remains colorless. From this it is obvious that pyrogallol is oxidized slowly by either hydrogen peroxid or oxygen and that its oxidation is merely accelerated, not initiated, by oxidizing enzymes.

[1] Schaer, E. Ueber den Einfluss alkalischer Substanzen auf Vorgänge der spontanen Oxydation. Archiv der Pharmazie, vol. 243, 1905, pp. 198–217.

DETAILED DESCRIPTION OF THE METHOD SUGGESTED.

The procedure of the actual measurements is as follows: The two-compartment oxidase apparatus (fig. 6) is clamped on the carriage in the thermostat. Eight cubic centimeters of fresh 0.1 of 1 per cent pyrogallol solution are measured into compartment *B* by means of the bulb *G*. Two cubic centimeters of plant juice are measured into compartment *A* from burette *F*. Basket *H* is charged with 1 cubic centimeter normal sodium hydrate solution. Stopcock *E* on the oxidase apparatus is closed, while *C* and *I* are left open. Then both doors and windows are tightly closed. The switch, figure 3, *e'*, controlling the permanent heating circuit, is turned on, and the double-throw switch *i'* is put into the upper position to increase the heat supplied. The four lamps *q*, *r*, *s*, and *t* are also thrown into series with the heater, as well as the switch *f'*, operating the temporary heating arrangement. In addition to these the switch *h'* is turned, in order to shorten the time necessary to heat the interior of the thermostat to the temperature desired. The establishment of this temperature is announced by a click of the relay *R*, and requires from one to two minutes. As soon as this happens switch *h'* is turned off, and a sufficient number of lamps to maintain the desired temperature are left in the permanent heating circuit, together with the lamps of the temporary heating circuit. A little experience with the apparatus makes it possible to have the conditions adjusted so that only one of the four lamps is automatically put on and off. Half an hour after the heating of the thermostat is begun the wooden panel and the two windows of the box are opened sufficiently to allow the introduction of the arm, and the stopcock *C* is quickly closed. Then the windows are again closed.

The shaking machine is set in motion by means of the rheostat, figure 2, *Rh*. The belt from the motor to the pulleys and the rheostat is adjusted to shake at a rate of five complete excursions of the carriage in 3.3 seconds, the magnitude of the excursions being 10 centimeters. The reaction begins as soon as the shaking is started. At intervals of 10 or 20 minutes the shaking is interrupted long enough to take readings of the manometers. The reaction is allowed to go on until no more oxygen is absorbed. The experiments as carried on so far have required about two hours each. The box is now opened, the oxidase bulb removed from the clamps, and the stopcocks *C* and *E* opened. The inner part of the ground joint with the basket is carefully taken out. The glass basket is quickly wiped on the outside, two drops of phenolphthalein solution are added to the sodium hydrate solution, and then the basket is at once fitted into the wide neck of the titration apparatus. The burette is filled with 0.1 normal sulphuric acid, and the liquid in the basket is titrated with constant, gentle agitation just to the point of the disappearance of

the red color. The burette is then read, three drops of Congo-red solution are placed in the basket, and the titration is continued until the bright red color disappears. From the difference between the two end points the quantity of carbon dioxid absorbed may be calculated.

EFFECT OF THE VARIABLE FACTORS INVOLVED IN THE METHOD ON THE TOTAL OXYGEN ABSORPTION.

In all of the experiments described in this bulletin the pressure readings on the manometer are given and these values reduced to the arbitrary volume of 150 cubic centimeters. An absolute unit of oxidase content, discussed farther on, is not made use of in the results given, since the results are only relative and have value only in proving the efficiency of the method. In each of the experiments 1 to 8, relating to variable factors, the volume of pyrogallol solution, 8 c. c., was the same, but the strength was varied, as stated in the several tables of results. The potato juice used was freshly prepared for each experiment, and, as it differed in each, different values were necessarily obtained from one experiment to another. The volume used, 2 c. c., was the same in each experiment, but in experiment 7 the concentration was varied, as stated in Table VII.

The contents of the glass absorption basket was the same (1 c. c. normal NaOH) in all experiments, but in experiment 8 the strength of the alkali solution was varied, as stated in Table VIII.

EFFECT OF VARYING THE CONCENTRATION OF PYROGALLOL.

Experiments 1 to 5, the results of which are shown in Tables I to V, were carried out to test the effect of varying the concentration of pyrogallol solution.

TABLE I.—*Readings of manometers as obtained from experiment 1.*[1]

Time of reading of manometer.	Elapsed time.	Temperature at the time of measurement.	Manometer readings, expressed in centimeters of mercury, using varying concentrations of the pyrogallol solution in apparatus—					
			Concentration 10 per cent.		Concentration 5 per cent.		Concentration 2.5 per cent.	
			No. 1.	No. 4.	No. 5.	No. 7.	No. 11.	No. 12.
	Minutes.	*° C.*						
11.45 a. m.	0	36.4	0	0	0	0	0	0
12.00 m.	15	36.4	− .50	− .60	− .52	− .55	− .70	− .65
12.15 p. m.	30	36.4	− .62	− .80	− .90	− .80	−1.00	−1.05
12.30 p. m.	45	36.4	− .85	− .95	− .98	− .80	−1.20	−1.10
1.30 p. m.	105	36.4	−1.25	−1.40	−1.32	−1.60	−1.70	−1.30
1.45 p. m.	120	36.4	−1.40	−1.60	−1.40	−1.60	−2.20	−1.40
2.00 p. m.	135	36.5	−1.50	−1.58	−1.45	−1.65	−3.20	−1.50
2.15 p. m.	150	36.4	−1.58	−1.80	−1.60	−1.80	[2]	−1.60
2.30 p. m.	165	36.5	−1.70	−1.80	−1.70	−1.80	[2]	−1.60
Final readings corrected to a volume of 150 c. c.			−1.62	−1.83	−1.72	−1.87		−1.42

[1] Put into the thermostat at 11.20 a. m.; shaking begun at 11.45 a. m.
[2] Pyrogallol solution splashed into the bulb.

TABLE II.—*Readings of manometers as obtained from experiment 2.*[1]

Time of reading of manometer.	Elapsed time.	Temperature at the time of measurement.	Manometer readings, expressed in centimeters of mercury, using varying concentrations of the pyrogallol solution in apparatus—					
			Concentration, 2.5 per cent.		Concentration, 1 per cent.		Concentration, 0.5 per cent.	
			No. 11.	No. 12.	No. 5.	No. 7.	No. 1.	No. 4.
	Minutes.	*° C.*						
10.30 a. m.	0	36.3	0	0	0	0	0	0
10.40 a. m.	10	36.3	−1.13	−1.07	−1.25	−1.20	−1.60	−1.20
11.00 a. m.	30	36.4	−1.95	−2.20	−2.40	−2.10	−2.80	−2.60
11.15 a. m.	45	36.4	−2.10	−2.40	−2.60	−2.35	−2.90	−2.80
11.30 a. m.	60	36.4	−2.20	−2 40	−2.72	−2.40	−3.00	−3.00
11.45 a. m.	75	36.4	−2.40	−2.75	−2.85	−2.55	−3.20	−3.00
12.00 m.	90	36.4	−2.50	−2.90	−3.00	−2.65	−3.25	−3.00
12.15 p. m.	105	36.4	−2.55	−3.00	−3.00	−2.68	−3.20	−3.05
Final readings corrected to a volume of 150 c. c			−2.43	−2.66	−3.04	−2.78	−3.06	−3.08

[1] Put into the thermostat at 10 a. m.; shaking begun at 10.30 a. m.

TABLE III.—*Readings of manometers as obtained from experiment 3.*[1]

Time of reading of manometer.	Elapsed time.	Temperature at the time of measurement.	Manometer readings, expressed in centimeters of mercury, using varying concentrations of the pyrogallol solution in apparatus—					
			No. 11, 16 per cent.	No. 12, 8 per cent.	No. 5, 4 per cent.	No. 7, 2 per cent.	No. 1, 1 per cent.	No. 4, 0.5 per cent.
	Minutes.	*° C.*						
2.20 p. m.	0	36.4	0	0	0	0	0	0
2.30 p. m.	10	36.4	− .78	−1.20	−1.20	−1.25	−1.60	−1.40
2.45 p. m.	25	36.4	−1.10	−1.60	−1.60	−1.75	−2.20	−1.80
3.00 p. m.	40	36.4	−1.20	−1.85	−1.85	−1.95	−2.30	−2.30
3.15 p. m.	55	36.4	−1.40	−2.20	−2.05	−2.10	−2.55	−2.40
3.30 p. m.	70	36.4	−1.50	−2.25	−2.25	−2.25	−2.60	−2.55
3.45 p. m.	85	36.4	−1.60	−2.38	−2.20	−2.25	−2.65	−2.60
4.00 p. m.	100	36.4	−1.70	−2.45	−2.30	−2.30	−2.75	−2.60
4.15 p. m.	115	36.4	−1.90	−2.60	−2.45	−2.45	−2.80	−2.55
4.30 p. m.	130	36.4	−2.05	−2.80	−2.50	−2.45	−2.95	−2.80
4.50 p. m.	150	36.4	−2.20	−2.80	−2.60	−2.50	−3.00	−2.70
Final readings corrected to a volume of 150 c. c.			−2.11	−2.48	−2.64	−2.60	−2.86	−2.76

[1] Put into the thermostat at 2 p. m.; shaking begun at 2.20 p. m.

TABLE IV.—*Readings of manometers as obtained from experiment 4.*[1]

Time of reading of manometer.	Elapsed time.	Temperature at the time of measurement.	Manometer readings, expressed in centimeters of mercury, using varying concentrations of the pyrogallol solution in apparatus—					
			No. 11, 0.8 per cent.	No. 12, 0.4 per cent.	No. 5, 0.2 per cent.	No. 7, 0.1 per cent.	No. 1, 0.05 per cent.	No. 4, 0.025 per cent.
	Minutes.	*°C.*						
9.45 a. m.	0	36.4	0	0	0	0	0	0
10.00 a. m.	15	36.4	− .60	− .95	− .45	− .00	+ .30	+ .30
10.15 a. m.	30	36.4	−1.02	−1.40	− .90	− .10	+ .25	+ .25
10.30 a. m.	45	36.5	−1.20	−1.58	−1.05	− .35	+ .10	+ .20
10.50 a. m.	65	36.4	−1.38	−1.70	−1.15	− .20	.00	+ .20
11.05 a. m.	80	36.4	−1.40	−1.65	−1.25	− .40	+ .10	+ .20
11.25 a. m.	100	36.4	−1.45	−1.65	−1.20	− .30	+ .10	+ .20
Final readings corrected to a volume of 150 c. c.			−1.39	−1.46	−1.22	− .31	+ .10	+ .20

[1] Put into the thermostat at 9.20 a. m ; shaking begun at 9.45 a. m.

TABLE V.—*Readings of manometers as obtained from experiment 5.*[1]

Time of reading of manometer.	Elapsed time.	Temperature at the time of measurement.	Manometer readings, expressed in centimeters of mercury, using varying concentrations of the pyrogallol solution in apparatus—					
			No. 1, 0.40 per cent.	No. 4, 0.35 per cent.	No. 5, 0.30 per cent.	No. 11, 0.25 per cent.	No. 7, 0.20 per cent.	No. 12, 0.15 per cent.
	Minutes.	*°C.*						
11.35 a. m.	0	36.4	0	0	0	0	0	0
11.50 a. m.	15	36.4	−1.00	− .70	− .70	− .45	− .40	− .20
12.05 p. m.	30	36.4	−1.20	− .95	− .95	− .65	− .50	− .20
1.05 p. m.	90	36.4	−1.70	−1.35	−1.50	−1.40	− .85	− .50
1.45 p. m.	130	36.5	−1.55	−1.20	−1.30	−1.20	− .80	− .65
2.05 p. m.	150	36.4	−1.60	−1.20	−1.25	−1.20	− .80	− .50
Final readings corrected to a volume of 150 c. c.			−1.52	−1.23	−1.27	−1.15	− .83	− .44

[1] Put into the thermostat at 11.15 a. m.; shaking begun at 11.35 a. m.

The results obtained in experiments 1 to 5 show that the concentration of the pyrogallol in the mixture (pyrogallol solution and potato juice) has no appreciable effect on the oxygen absorption provided the concentration is above a certain lower limit. This lower limit, as experiment 5 plainly shows, is reached when 8 cubic centimeters of 0.25 of 1 per cent pyrogallol solution are added to 2 cubic centimeters of potato juice or when the concentration of the pyrogallol is 0.20 of 1 per cent under the conditions of the experiment. No attempt was made in the course of the work here described to determine exactly the smallest quantity of pyrogallol required; the experiments carried out had the sole purpose of testing the degree of concentration of pyrogallol solution necessary to obtain comparable results.

From experiment 3 it is apparent that very great concentrations of pyrogallol, such as 16 per cent, have a slight retarding action on the oxidation. This is especially noticeable in the rate with which the end point is reached.

On the strength of the results of these experiments it was decided to use, at least for the present, a 1 per cent pyrogallol solution in all the experiments to be made.

COMPARATIVE EFFECTIVENESS OF FRESH AND OF OLD PYROGALLOL SOLUTIONS.

Pyrogallol solutions on standing assume first a yellow, then an orange, then cherry red, and finally a deep red color. One of the solutions here used had stood for about a year and was very deep red in color. In experiment 6 it was compared with a fresh, colorless solution.

TABLE VI.—*Readings of manometers as obtained from experiment 6.*[1]

Time of reading of manometer.	Elapsed time.	Temperature at the time of measurement.	Manometer readings, expressed in centimeters of mercury, using varying proportions of fresh and of old pyrogallol solution in apparatus—			
			No. 5, 1 per cent old.	No. 7, 1 per cent fresh.	No. 11, 0.1 per cent old.	No. 12, 0.1 per cent fresh
	Minutes.	*° C.*				
9.35 a. m.	0	36.4	0	0	0	0
9.45 a. m.	10	36.4	−.80	−.80	−.40	−.60
10.00 a. m.	25	36.4	−1.50	−1.45	−.70	−.80
10.15 a. m.	40	36.4	−1.80	−1.75	−.80	−.90
10.35 a. m.	60	36.4	−1.80	−1.85	−.85	−.95
Final readings corrected to a volume of 150 c. c.			−1.82	−1.92	−.79	−.83

[1] Put into the thermostat at 9 a. m.; shaking begun at 9.25 a. m. Rate of shaking 5 complete excursions in 3 seconds.

Experiment 6 was undertaken to determine whether it is necessary to prepare a fresh pyrogallol solution at the beginning of each experiment. As the experiment shows, the very old solution gives the same result as that freshly prepared and therefore no precautions need be taken in this respect. The difference between the results of the first series (Nos. 5 and 7) and last (Nos. 11 and 12) is due to the low concentration of the pyrogallol in the second series. (See experiments 4 and 5.)

EFFECT OF VARYING THE CONCENTRATION OF POTATO JUICE.

Experiment 7 was conducted to determine the effect of varying the concentration of the potato juice used.

TABLE VII.—*Readings of manometers as obtained from experiment 7.*[1]

Time of reading of manometer.	Elapsed time.	Temperature at the time of measurement.	Manometer readings, expressed in centimeters of mercury, using potato juice diluted in varying degrees in apparatus—					
			No dilution.		Dilution one-half.		Dilution three-fourths.	
			No. 1.	No. 4.	No. 5.	No. 7.	No. 11.	No. 12.
	Minutes.	° *C.*						
1.30 p. m.	0	36.3	0	0	0	0	0	0
1.40 p. m.	10	36.4	−1.00	− .90	− .60	− .40	− .20	− .30
1.50 p. m.	20	36.4	−1.40	−1.20	− .80	− .60	− .20	− .30
2.00 p. m.	30	36.4	−1.55	−1.42	−1.00	− .20	− .22	− .35
2.10 p. m.	40	36.4	−1.62	−1.55	−1.08	− .80	− .30	− .40
2.20 p. m.	50	36.5	−1.76	−1.70	−1.18	− .85	− .35	− .40
2.30 p. m.	60	36.4	−1.80	−1.80	−1.25	− .90.	− .35	− .42
2.40 p. m.	70	36.4	−1.80	−1.82	−1.40	− .90	− .40	− .50
2.50 p. m.	80	36.4	−1.82	−1.83	−1.50	− .94	− .40	− .50
3.00 p. m.	90	36.4	−1.82	−1.85	−1.60	− .95	− .40	− .55
Final readings corrected to a volume of 150 c. c.			−1.73	−1.89	(2)	− .97	− .38	− .48

[1] Put into the thermostat at 1 p. m.; shaking begun at 1.30 p. m. Rate of shaking, 5 complete excursions in 3 seconds.
[2] Pyrogallol splashed into the basket.

Experiment 7 shows that the total oxygen absorption is at least approximately proportional to the quantity of potato juice present. What the actual relationship is between the concentration of potato juice and the quantity of oxygen absorbed is for later determination.

EFFECT OF CONCENTRATION OF ALKALI IN THE ABSORPTION BASKET.

It was necessary to determine the strength of the alkali solution required to insure prompt and complete absorption of the carbon dioxid produced during the experiments. For this purpose experiment 8 was carried out.

TABLE VIII.—*Readings of manometers as obtained from experiment 8.*[1]

Time of reading of manometer.	Elapsed time.	Temperature at the time of measurement.	Manometer readings, expressed in centimeters of mercury, using varying strengths of alkali in apparatus—					
			No. 1, alkali 2.5 normal.	No. 4, alkali normal.	No. 5, alkali 0.5 normal.	No. 7, alkali 0.25 normal.	No. 11, alkali 0.1 normal.	No. 12, alkali none.
	Minutes.	° *C.*						
2.40 p. m.	0	36.4	0	0	0	0	0	0
3.00 p. m.	20	36.4	−1.70	−1.10	−1.20	−1.15	−1.20	− .80
3.30 p. m.	50	36.4	−2.05	−1.60	−1.85	−1.65	−1.60	− .80
4.00 p. m.	80	36.4	−2.20	−1.70	−2.00	−1.90	−1.80	−1.00
4.20 p. m.	100	36.4	−2.05	−1.70	−2.05	−1.90	−1.80	− .90
Final readings corrected to a volume of 150 c. c.			−2.10	−1.73	−2.08	−1.98	−1.73	− .80

[1] Put into the thermostat at 2.25 p. m.; shaking begun at 2.40 p. m. Rate of shaking, 5 complete excursions in 3.3 seconds. Concentration of pyrogallol solution 1 per cent.

As these results show, it is necessary to use at least 0.25 normal solution of sodium hydrate to make sure of the complete removal of the carbon dioxid formed during the oxidation of the pyrogallol. To be certain of an excess of alkali, 1 cubic centimeter of a normal solution was used in all these experiments. There seems to be no doubt that under the conditions the absorption of the carbon dioxid from the atmosphere of the flasks is practically complete. This presumption is borne out by the fact that in all of the experiments the reaction comes to completion within a few hours. If a measurable amount of carbon dioxid were unabsorbed in the oxidase apparatus, the pressure as indicated by the manometer would diminish as the shaking is continued until practically all of the carbon dioxid is absorbed.

EFFECT OF VARYING THE RATE OF SHAKING.

As can be seen from the experiments cited, the oxidase apparatus was shaken at a rate of five complete excursions in 3 to 3.1 seconds. Under these conditions in some cases a small amount of pyrogallol splashed into the alkali in the small glass basket (No. 11, experiment 1; and No. 5, experiment 7). The splashing became noticeable at once by an increased rate of oxygen absorption and the failure of the absorption to come to completion. It is impossible to overlook such an error, for the reason that no experiment is taken into account unless the diminution of pressure comes to a definite end in the course of a few hours. To avoid accidents due to the splashing of pyrogallol into the basket, the rate of shaking was reduced to five complete excursions in 3.3 seconds. Under these conditions, as may be seen, no difficulty due to splashing was experienced.

EFFECT OF VARYING TEMPERATURE.

The temperature in the thermostat varied, as experiments 1 to 8 show, no more than 0.1 of a degree, and it is certain that the maximal variations within the oxidase apparatus were less than that. Since the pressure is directly proportional to the absolute temperature, a rise of 0.1 of a degree at 36.4° C. will involve an increase of pressure of $\frac{76}{309.4 \times 10}$ i. e., 0.025 of a centimeter of mercury. This is not greater than the errors involved in the measurements of the pressure existing within the oxidase apparatus.

EFFECT OF SHAKING ON THE ACTIVITY OF THE POTATO JUICE.

Since it is known from the work of Meltzer, Schmidt-Nielssen, and others that many of the enzymes lose their activity on vigorous shaking, it seemed advisable to see whether the potato juice loses its activity to any extent on account of the shaking during the experiments. This was hardly to be expected, since the rate of shaking employed was never very vigorous.

To test the point in question, three experiments (9, 10, and 11) were carried out as follows: Oxidase apparatus were clamped to the carriage and the baskets charged with 1 cubic centimeter of normal sodium hydrate. Into each apparatus were put 2 cubic centimeters of potato juice and 6 cubic centimeters of water. Two cubic centimeters of a 4-per cent pyrogallol solution were placed in each of the graduated pipettes. In this fashion, after mixing, the usual dilutions were obtained. The pyrogallol solution was run into one apparatus just before the shaking was begun, into another some time later, into a third still later, and so on.

TABLE IX.—*Manometer readings obtained from experiment 9.*[1]

Time of reading of manometer.	Elapsed time.	Temperature at the time of measurement.	Manometer readings, expressed in centimeters of mercury, in apparatus—					
			No. 1.	No. 4.	No. 5.	No. 7.	No. 11.	No. 12.
	Minutes.	° *C.*						
10.30 a. m.	0	36.4	[2] 0	0	0	0	0	0
10.45 a. m.	15	36.4	−1.30	0	0	0	0	0
11.00 a. m.	30	36.4	−1.82	[2] 0	0	0	0	0
11.15 a. m.	45	36.4	−1.95	− .85	0	0	0	0
11.30 a. m.	60	36.4	−2.10	−1.10	[2] 0	0	0	0
11.45 a. m.	75	36.4	−2.15	−1.25	− .45	0	0	0
12.00 m.	90	36.4	−2.30	−1.35	− .70	[2] 0	0	0
12.15 p. m.	105	36.4	−2.32	−1.48	− .90	− .75	0	0
12.30 p. m.	120	36.4	−2.25	−1.45	− .90	−1.05	[2] 0	0
1.00 p. m.	150	36.4	−2.45	−1.60	−1.10	−1.20	−1.20	[2] 0
1.30 p. m.	180	36.4	−2.45	−1.70	−1.15	−1.30	−1.50	−1.40
2.00 p. m.	210	36.5	−2.45	−1.72	−1.20	−1.35	−1.60	−1.50
2.30 p. m.	240	36.5	−2.60	−1.90	−1.30	−1.50	−1.80	−1.60

[1] Put into the thermostat at 10 a. m.; shaking began at 10.30 a. m. Rate of shaking, 5 complete excursions in 3.3 seconds.
[2] Solution run into apparatus.

Unfortunately this experiment had to be discontinued at 2.30 p. m. Nevertheless it shows a fairly good agreement between all but the first two of the series.

TABLE X.—*Manometer readings obtained from experiment 10.*[1]

Time of reading of manometer.	Elapsed time.	Temperature at the time of measurement.	Manometer readings, expressed in centimeters of mercury, in apparatus—					
			No. 1.	No. 4.	No. 5.	No. 7.	No. 11.	No. 12.
	Minutes.	° *C.*						
10.00 a. m.	0	36.4	[2] 0	0	0	0	0	0
10.30 a. m.	30	36.5	−1.80	[2] 0	0	0	0	0
11.00 a. m.	60	36.4	−2.10	−1.25	[2] 0	0	0	0
11.30 a. m.	90	36.5	−2.35	−1.65	−1.10	[2] 0	0	0
12.00 m.	120	36.5	−2.42	−1.70	−1.40	−1.00	[2] 0	0
12.30 p. m.	150	36.4	−2.70	−1.90	−1.50	−1.35	−1.20	[2] 0
1.00 p. m.	180	36.4	−2.80	−2.00	−1.80	−1.50	−1.50	−1.40
1.30 p. m.	210	36.4	−2.75	−2.10	−1.70	−1.50	−1.50	−1.70
2.00 p. m.	240	36.4	−2.80	−2.20	−1.90	−1.75	−1.80	−1.80
2.30 p. m.	270	36.4	−2.80	−2.20	−1.90	−1.75	−1.80	−1.90
Final readings corrected to a volume of 150 c. c.			−2.67	−2.24	−1.93	−1.82	−1.73	−1.68

[1] Put into the thermostat at 9.40 a. m.; shaking began at 10 a. m. Rate of shaking, 5 complete excursions in 3.4 seconds.
[2] Solution run into apparatus.

Experiment 11 was a repetition of experiments 9 and 10, with the reactions in the various oxidase apparatus initiated in a reverse order.

TABLE XI.—*Manometer readings obtained from experiment 11.*[1]

Time of reading of manometer.	Elapsed time.	Temperature at the time of measurement.	Manometer readings, expressed in centimeters of mercury, in apparatus—					
			No. 1.	No. 4.	No. 5.	No. 7.	No. 11.	No. 12.
	Minutes.	° *C.*						
12.10 p. m.	0	36.4	0	0	0	0	0	[2] 0
12.25 p. m.	15	36.3	0	0	0	0	[2] 0	−1.10
12.40 p. m.	30	36.4	0	0	0	[2] 0	− .55	−1.60
1.00 p. m.	50	36.4	0	0	[2] 0	− .60	−1.00	−1.60
1.30 p. m.	80	36.4	0	[2] 0	− .50	− .80	−1.10	−1.80
2.00 p. m.	110	36.4	[2] 0	− .80	− .80	− .80	−1.20	−1.35
2.30 p. m.	140	36.4	− .50	− .90	− .90	−1.00	−1.20	−2.00
3.00 p. m.	170	36.4	− .70	−1.00	− .70	−1.05	−1.30	−2.05
3.30 p. m.	200	36.4	− .80	−1.05	− .95	−1.05	−1.30	−2.20
4.00 p. m.	230	36.4	− .80	−1.05	−1.00	−1.05	−1.25	−2.30
4.30 p. m.	260	36.4	− .90	−1.10	−1.08	−1.15	−1.30	−2.20
Final readings corrected to a volume of 150 c. c.			− .86	−1.12	−1.09	−1.19	−1.25	−1.95

[1] Put into the thermostat at 11.30 a. m.; shaking begun at 12.10 p. m. Rate of shaking, 5 complete excursions in 3.3 seconds.
[2] Solution run into apparatus.

These results bring out a very remarkable fact. If the potato juice is shaken for 15 to 30 minutes before the addition of the oxidizable substance, its oxidizing power is reduced to about half its original value. On longer shaking very little effect is noticeable. Potato juice shaken for 2½ hours gives nearly the same result as that

shaken only one-half hour. Whatever change the juice undergoes takes place in the first half hour of the experiment. The activity after this period is still quite considerable and does not suffer any appreciable loss on further shaking for two or three hours, which is the maximum duration of the measurements. This indicates that two phases of the process are dealt with, each one of which may be measured separately. In order to get the total oxidizing effect of the plant juice, it is necessary to take the measurements from the time the shaking is begun.

Experiments 1 to 11 very clearly point out the conditions under which the experiments must be carried out in order to obtain comparable results. The details of the method are based on these experiments and are described in an earlier part of the paper. The experiments also show that by means of this method it is possible to obtain quite accurate and reliable results, as shown by the numerous parallel experiments. It is true that in some of the duplicate experiments, especially at the beginning of the investigation, there are differences in the final results of from 2 to 3 millimeters or even more (experiments 11 and 12), but these differences become smaller as the work advances and the investigator's familiarity with the apparatus increases.

PRACTICAL APPLICATION OF THE METHOD TO THE STUDY OF THE CURLY-TOP OF BEETS.

The Division of Cotton and Truck-Crop Diseases of the Bureau of Plant Industry, U. S. Department of Agriculture, for some years has been investigating the curly-top of sugar beets.[1] Through the courtesy of Mr. W. A. Orton and Mr. H. B. Shaw, the writer was able to obtain for experimental purposes fresh samples of sugar-beet leaves affected by this condition to a striking degree, and also samples of normal beet leaves. All of the beets the leaves of which were examined were grown in a greenhouse, and therefore were subjected to practically uniform conditions. The leaves were treated the same as the potato peelings. They were crushed in a meat chopper and the juice pressed out through a piece of silk cloth. Of the two sets of oxidase apparatus used in these experiments, *a* had a volume of 151 cubic centimeters and *b* a volume of 140 cubic centimeters.

With the exception of experiment 15, in each of experiments 12 to 17 the volume of pyrogallol solution used was 8 c. c. and its concentration was 1 per cent. The volume of beet-leaf juice was 2 c. c., and the alkali used was 1 c. c. of normal NaOH. In experiment 15 the

[1] Shaw, Harry B. The Curly-Top of Beets. Bulletin 181, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1910.

conditions in apparatus *b* were the same as in the other experiments, while in apparatus *a* the 8 c. c. of pyrogallol were replaced by 8 c. c. of distilled water.

TABLE XII.—*Manometer readings obtained from experiment 12.*[1]

Time of reading of manometer.	Elapsed time.	Manometer readings, expressed in centimeters of mercury, in apparatus—	
		a. Normal.	*b.* Normal.
	Minutes.		
2.55 p. m	0	0	0
3.15 p. m	20	− .52	− .27
3.35 p. m	40	− .95	− .69
3.45 p. m	50	−1.09	− .91
3.55 p. m	60	−1.08	−1.00
4.05 p. m	70	−1.15	−1.10
4.15 p. m	80	−1.15	−1.15
Final readings corrected to a volume of 150 c. c		−1.16	−1.07

[1] Put into the thermostat at 2.30 p. m.; shaking begun at 2.55 p. m. Rate of shaking, 5 complete excursions in 3.5 seconds.

This experiment shows a very good agreement between the final results in the two series. The determination of the carbon dioxid absorbed by the alkali in the glass baskets was less successful, since a little too much acid was used in apparatus *b* for the final neutralization.

Carbon dioxid.—Number of cubic centimeters of 0.1 normal sulphuric acid required to neutralize to phenolphthalein, alkali in basket of apparatus *a*, 8.85. Number of additional cubic centimeters of 0.1 normal sulphuric acid required to neutralize to Congo red, 0.68. Grams of carbon dioxid absorbed, 0.00150.

TABLE XIII.—*Manometer readings obtained from experiment 13.*

Time of reading of manometer.	Elapsed time.	Manometer readings, expressed in centimeters of mercury, using either beet-leaf juice or distilled water in apparatus—	
		a, Pathological beet-leaf juice.	*b*, Distilled water.
	Minutes.		
10.50 a. m	0	0	0
11.35 a. m	45	−3.29	+ .50
12.00 m	70	−4.60	+ .45
12.20 p. m	90	−5.00	+ .45
12.20 to 3 p. m.[1]			
3.00 p. m	250	−5.60	+ .60
4.05 p. m	315	−5.57	+ .55
Final reading corrected to a volume of 150 c. c		−5.61	

[1] Experiment interrupted on account of a broken belt.

Carbon dioxid.—Number of cubic centimeters of 0.1 normal sulphuric acid required to neutralize to phenolphthalein, alkali in basket of apparatus *a*, 6.76. Number of additional cubic centimeters of 0.1 normal sulphuric acid required to neutralize to Congo red, 2.29. Grams of carbon dioxid absorbed, 0.00504. Number of cubic centimeters of 0.1 normal sulphuric acid required to neutralize to phenolphthalein, alkali in basket of apparatus *b*, 9.52. Number of additional cubic centimeters of 0.1 normal sulphuric acid required to neutralize to Congo red, 0.10.

TABLE XIV.—*Manometer readings obtained from experiment 14.*

Time of reading of manometer.	Elapsed time.	Manometer readings, expressed in centimeters of mercury, using either pathological or normal beet-leaf juice in apparatus—	
		a, Pathological.	*b*, Normal.
	Minutes.		
3.15 p. m.	0	0	0
3.30 p. m.	15	−1.28	− .45
3.40 p. m.	25	−2.16	− .75
3.55 p. m.	40	−3.20	− .96
4.05 p. m.	50	−3.82	−1.04
4.15 p. m.	60	−4.20	−1.17
4.25 p. m.	70	−4.35	−1.18
4.40 p. m.	85	−4.27	−1.18
Final readings corrected to a volume of 150 c. c.		−4.30	−1.10

Carbon dioxid.—Number of cubic centimeters of 0.1 normal sulphuric acid required to neutralize to phenolphthalein, alkali in basket of apparatus *a*, 7.70. Number of additional cubic centimeters of 0.1 normal sulphuric acid required to neutralize to Congo red, 1.85 Grams of carbon dioxid absorbed, 0.00407. Number of cubic centimeters of 0.1 normal sulphuric acid required to neutralize to phenolphthalein, alkali in basket of apparatus *b*, 9.01. Number of additional cubic centimeters of 0.1 normal sulphuric acid required to neutralize to Congo red, 0.64. Grams of carbon dioxid absorbed, 0.00141.

TABLE XV.—*Manometer readings obtained from experiment 15.*

Time of reading of manometer.	Elapsed time.	Manometer readings, expressed in centimeters of mercury, in apparatus.	
		a. Normal + 8 c. c. distilled water.	*b.* Normal + 8 c. c. pyrogallol.
	Minutes.		
11.05 a. m.	0	0	0
11.30 a. m.	25	0	− .70
11.55 a. m.	50	+ .20	−1.25
12.15 p. m.	70	+ .20	−1.21
12.30 p. m.	85	+ .20	−1.25
Final reading corrected to a volume of 150 c. c			−1.17

Carbon dioxid.—Number of cubic centimeters of 0.1 normal sulphuric acid required to neutralize to phenolphthalein, alkali in basket of apparatus *a*, 9.30. Number of additional cubic centimeters of 0.1 normal sulphuric acid required to neutralize to Congo red, 0.20. Grams of carbon dioxid absorbed, 0.00044. Number of cubic centimeters of 0.1 normal sulphuric acid required to neutralize to phenolphthalein, alkali in basket of apparatus *b*, 8.80. Number of additional cubic centimeters of 0.1 normal sulphuric acid required to neutralize to Congo red, 0.75. Grams of carbon dioxid absorbed, 0.00165.

TABLE XVI.—*Manometer readings obtained from experiment 16.*

Time of reading of manometer.	Elapsed time.	Manometer readings, expressed in centimeters of mercury, using either pathological or normal beet-leaf juice in apparatus—	
		a. Pathological.	*b.* Normal.
	Minutes.		
4.00 p. m.	0	0	0
4.15 p. m.	15	− .40	− .40
4.30 p. m.	30	−1.40	−0.75
4.45 p. m.	45	−2.00	−0.88
5.00 p. m.	60	−2.22	−1.15
5.15 p. m.	75	−2.43	−1.18
5.30 p. m.	90	−2.60	−1.20
5.45 p. m.	105	−2.50	−1.25
6.00 p. m.	120	−2.70	−1.27
Final readings corrected to a volume of 150 c. c.		−2.72	−1.19

Carbon dioxid.—Number of cubic centimeters of 0.1 normal sulphuric acid required to neutralize to phenolphthalein, alkali in basket of apparatus *a*, 8.20. Number of additional cubic centimeters

of 0.1 normal sulphuric acid required to neutralize to Congo red, 1.22. Grams of carbon dioxid absorbed, 0.00312. Number of cubic centimeters of 0.1 normal sulphuric acid required to neutralize to phenolphthalein, alkali in basket of apparatus *b*, 8.85. Number of additional cubic centimeters of 0.1 normal sulphuric acid required to neutralize to Congo red, 0.85. Grams of carbon dioxid absorbed, 0.00187.

TABLE XVII.—*Manometer readings obtained from experiment 17.*

Time of reading of manometer.	Elapsed time.	Manometer readings, expressed in centimeters of mercury, using either pathological or normal beet-leaf juice in apparatus—	
		a. Normal.	*b*. Pathological.
	Minutes.		
2.10 p. m.	0	0	0
2.40 p. m.	30	−.82	−.90
3.00 p. m.	50	−1.28	−1.46
3.15 p. m.	65	−1.20	−1.50
3.35 p. m.	85	−1.27	−1.65
3.50 p. m.	100	−1.20	−1.62
Final readings corrected to a volume of 150 c. c.		−1.21	−1.51

Experiments 14 to 17 show a very striking difference between the juice of the normal and that of the diseased beet leaves. In all of the experiments the oxidase content as indicated by the oxygen absorption of the pyrogallol in the presence of the juice is markedly greater in the diseased than in the healthy leaves. The oxidase content of the normal leaves seems to be fairly constant, while the juice of the leaves of beets with curly-top shows wide variations. The leaves used in experiment 13 give about five times as high a figure as normal leaves, while the leaves chosen in experiment 17 show a variation of only 25 per cent from the normal. It is very interesting to note that the deviation in oxidase content of the pathological leaves, as measured by the method described, runs parallel with the appearance of the leaves. The plants used in experiment 13 showed very marked signs of curly-top, the leaves being small and shriveled and the hairy roots abundant, while the juice of the diseased beet used in experiment 17, which showed a relatively low oxidase content although higher than normal, had only a slight curling of the leaves.

It is fully realized that these experiments are subject to the criticism that the juices of the two sets of leaves as prepared for the experiments may not be comparable. It may be merely an expression of the fact that one set of leaves is richer in cells than the other, or it

might be that one is richer in water or in cellulose than the other. It is hoped to settle these questions by paralleling the oxidase determinations with determinations of water, nitrogen, etc. Since the pathological leaves had in some cases more than three times the oxygen absorbing power of the controls, it seems hardly possible that this sympton can depend upon mere differences in the gross composition of the leaves.

DISCUSSION OF RESULTS.

The main object of this bulletin is to describe a new method for the estimation of oxidases in plant juices. The method has been tried with a number of samples of potato juice and found to give results in good agreement. There are still slight deviations between the results of duplicate determinations, and efforts are being made at present to reduce these to a minimum. The main source of error lies in the rise of pressure within the oxidase apparatus as soon as the shaking is begun. This rise of pressure is also obtained when distilled water is used instead of pyrogallol and potato juice. It is probably due to the formation of water particles in the gas above the liquid in the oxidase apparatus thereby increasing the specific gravity of the gas. The difficulty can be overcome in several different ways. One could interrupt the shaking in the beginning of the experiment every few minutes to make a reading. The end result would then be based on the difference between the highest pressure reading obtained and the reading made when the absorption is complete. Or, it is possible to make blank determinations without the use of potato juice and to correct the readings in all the experiments by adding to them the reading obtained in the blank. A third mode of obviating the difficulty would be to open one of the stopcocks of the oxidase apparatus for a moment when the maximum pressure is attained in the beginning of the experiment, so that the pressure within the apparatus may be reduced to atmospheric pressure. The latter method would be the least desirable since it involves opening the window of the box and therefore additional errors due to slight fall in temperature are likely to occur. Furthermore, this procedure would not be applicable to experiments in which the oxidation is rapid.

A number of experiments have been carried out in which the influence of the variable factors of the method on the final result has been studied. Incidentally several very interesting facts came to light in the course of these experiments. The most important of these perhaps is the fact that only a very definite and limited quantity of oxygen is absorbed by pyrogallol in the presence of a definite quantity of potato juice within a short period of time, say, two or three hours. Oxidation of the pyrogallol will proceed after that time

but at a rate which is not measurable under the conditions of the experiment.

The concentration and total quantity of pyrogallol present is without effect on the final result, provided the pyrogallol is in excess. This observation can be explained by either one of two assumptions. The quantity of plant juice used in the experiments is either capable of rendering a definite quantity of oxygen active enough to oxidize the corresponding amount of pyrogallol, or it can combine with a definite quantity of pyrogallol and transform it into more easily oxidizable material. Excess of the oxidizable material above that corresponding to the oxidase in the plant juice present is therefore without effect on the final result obtained. Within the limits of the experiments, the amount of the chemical change is directly proportional to the concentration of the oxidase present, all other factors remaining the same; doubling the volume of potato juice doubles the volume of oxygen absorbed.

Chodat,[1] working with Lactarius juice, could not confirm the law of direct proportionality which he and Bach propounded for the action of peroxidase, but obtained experimental indications that the discrepancy is due to the inadequacy of his technique.

These facts are in contradiction to our conception of enzyme action in general. We are accustomed to look at enzymes as catalytic agents, quite analogous in their mode of action to the inorganic catalysts. If the substances in the potato juice which are responsible for the rapid absorption of oxygen by the pyrogallol were enzymes in the accepted sense of the word, one would expect small quantities of the juice to bring about the oxidation of relatively large quantities of pyrogallol and that the oxidation would continue as long as pyrogallol and free oxygen are present, or until the activity of the juice is lost by deterioration. In the reaction discussed in this article the process comes to completion when only a small definite portion of the pyrogallol is oxidized and the oxygen is still in abundance.

It seems, therefore, that the oxidase in potato juice, which accelerates the oxidation of pyrogallol by atmospheric oxygen, is not an enzyme in the customary sense of the word, but rather a substance entering directly into the reaction and being destroyed in the course of the same.

This simultaneous oxygen transfer and self-destruction can be imagined to take place in at least two ways. It is conceivable that the active oxidase molecules are capable of combining with a certain number of oxygen atoms. This combination would then have an oxidation potential high enough to oxidize pyrogallol. In the pres-

[1] Chodat, R. Mode de l'Action de l'Oxydase. Archives des Sciences Physiques et Naturelles, vol. 19, p. 501.

ence of pyrogallol the oxygen is torn off with such violence that the whole oxidase molecule is disrupted. If this were the case, the oxygen absorption would be independent of the presence of pyrogallol, unless the combination between the oxygen and the oxidase were a balanced reaction.

The second assumption is that the oxidase acts on the pyrogallol. It would combine with it to form an easily oxidizable compound, the rate of oxidation of which would depend primarily on the concentration of the oxygen in solution. Every molecule of the easily oxidizable compound combines with one or more atoms of oxygen and simultaneously is decomposed in such a way that the originally active oxidase is destroyed or otherwise injured. The writer inclines to this view as a provisional working hypothesis. It is the plan to carry out a series of experiments to test the correctness of the assumptions here made.

With the exception of a few isolated cases, there exists no conception of what the composition of the so-called oxidase is. There are only theories as to their mode of action, and on account of the diversity of the reactions they accelerate or bring about, as the case may be, we have not even a satisfactory definition to cover all of them. A starting point in their exact study must be made, and it seemed to the writer necessary to take one type of reaction after another and correlate them, if possible, at the end. In this paper only the oxidation of pyrogallol by atmospheric oxygen has been considered, and the method here worked out serves simply as a measure of the weight of oxygen that pyrogallol is capable of taking up in neutral aqueous solutions, due to the interaction of a certain volume of plant juice.

After the study of pyrogallol has been exhausted from this point of view, other compounds, such as hydrochinone, thymol, tannic acid, various sugars, etc., will be used. Then the reaction of the medium will be varied. It is hoped that on the basis of these experiments the oxidases may be classified.

Since it is desirable to express the strength of a juice in terms of some standard, the writer proposes as a unit for future experiments an oxidase solution of such strength that 1 liter of it will be capable of bringing about the consumption by pyrogallol of the equivalent of 1 gram of hydrogen—i. e., a unit of 8 grams of oxygen. This unit of "strength" may not have any relation to the rate of the absorption, as it refers here explicitly only to the total amount absorbed. It is customary to measure the "activity" of an enzyme by the rate of action. It is an interesting question for future investigation whether the strength of an oxidase solution as expressed by this proposed standard is proportional to the rate at which the absorption takes place.